eDNA Field Sampling Abbreviated Protocol

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This protocol is intended to be a field reference for the collection of eDNA samples. It does not cover the concepts of avoiding contamination and sampling site selection. Refer to the current General Technical Report (GTR) for the full protocol and troubleshooting guide.

Before Leaving for the Field

- Read full eDNA protocol (GTR: Carim et al. 2016) and the most recent field advisory.
- Charge pump batteries and check contents of duffel bag and pump case.
- Remember to clean pump, tubing, and bucket if moving between watersheds.

Reminders to Avoid Contamination

- Always keep personnel and gear downstream of sampling locations.
- Move upstream between sites while collecting samples.
- If you suspect that any gloves, forceps, filter cups, samples, etc. have become contaminated, immediately discard the item and start over with a new kit.

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eDNA Sample Collection

1. Select an appropriate sampling site for your project objectives.
    - Sample downstream of suitable habitat. See GTR for details.
    - Beware of sampling in an eddy, backflow, splash pool, or anywhere in which flows might transport DNA from you onto the sample filter.
    - Make sure work area is free from hazardous obstacles.
2. Place pump and battery in stable area. Connect pump to battery using power cord.
3. Load tubing into the pump. Lift the lever on the pump head, load the tubing, then lower the lever. Follow the tubing orientation marked on pump and place outflow end of the tubing into the bucket.
4. Ensure pump speed is set to FAST and pump direction is set to FORWARD. Test suction from the filter adapter on your hand. If necessary to set down, place adaptor end of tubing down in an area where dirt/debris will not enter and clog the tubing.
5. Remove sampling kit from the “clean” white bag and immediately close the “clean” bag. Keep unused kits separate from working area to prevent contamination between sites.
6. Put on gloves from kit. Once wearing gloves do not touch anything that may be contaminated with DNA from outside the site (you, your pack, the pump, etc.).
7. Remove the packaged filter cup from the gallon kit bag. Without touching the filter cup, open the bag and rotate the filter cup within the Ziploc so that the blue base is facing towards the opening.
8. While holding the filter cup through the Ziploc, attach it to the tubing by pressing the blue base onto the tubing adapter. Do not fully remove the cup from packaging until ready to collect the sample. See photos on back.
    - If sampling by yourself, the hand that holds the tubing in now “dirty” and should not come into contact with the filter cup or “clean” sampling supplies for the remainder of sampling. See GTR for further details.

9. Turn the pump on. While holding the filter cup by the adapter, lower the cup into the stream, keeping it in front of you and pointing upstream. In shallow streams, the tubing can be carefully lodged under a rock during filtering to hold it in place for the duration of filtering. Be sure not to pass your hand in front of the filter cup or dislodge rocks upstream of the filter cup.
    - To prevent contamination, keep all equipment and yourself downstream of the filter cup. Do not hold the cup by anything other than the adapter.
10. Filter 5 liters of water, measured by the 5 L mark in the outflow bucket. Filtering generally takes 8–10 minutes, but can take up to 20 minutes. If the amount of water being filtered slows considerably, the filter may be clogged.
    - If filter is clogged, follow protocol until step 15. Record the volume of filtered water on filter Ziploc. Continue from step 7 with a new filter until a total of 5 L of water has been filtered. However, **do not exceed 3 filters per site**, even if you do not reach 5 L.
11. Lift the cup upwards and away from the stream. Continue to run the pump for ~30 seconds to dry the filter.
12. Remove the cup from filter holder (squeeze and pull off, do not twist).
13. With the sterile forceps, fold the filter in quarters, with the filtering side in. **Do not touch the forceps to anything other than the filter.**
14. Place folded filter into the Ziploc containing silica beads. Do not allow anything to come in contact with the inside of the silica bag except for filter (watch contact with hands). Make sure filter is touching the silica beads, remove excess air, and seal completely.
15. For each filter, clearly label the Ziploc with the date, stream name, site, GPS coordinates, volume filtered, and your initials.
16. Turn off pump before placing tubing on the ground to avoid clogging it with dirt/debris.
17. Return filter cup and forceps back into the gallon Ziploc, and place in black “used equipment” bag. Discard gloves and other Ziplocs directly into black bag.
18. Fill out the envelope with the same sample information as recorded in step 15. In addition, please add the total number of filters used at the site and their respective volumes. Duplication of this data from the Ziploc is necessary to prevent data loss.
19. Seal the Ziploc(s) in the labeled envelope and store separately from sampling materials to prevent cross-contamination. Store out of direct sunlight, free from excessive weight or handling, and extreme heat (i.e. don’t leave in your field truck).
20. Discard the water from the outflow bucket.

GTR Citation: Carim, Kellie J.; McKelvey, Kevin S.; Young, Michael K.; Wilcox, Taylor M.; Schwartz, Michael K. 2016. A protocol for collecting environmental DNA samples from streams. Gen. Tech. Rep. RMRS-GTR-355. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 18 p.